

**Effect of early rehabilitation using an active/passive cycling device
on muscle wasting in the critically ill:**

A randomised controlled pilot study

Short Title – Muscle Wasting in the Critically Ill.

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Abbreviations and definitions

6MWT	Six minute walk test
APACHE II	Acute Physiology And Chronic Health Evaluation II
BMI	Body Mass Index
CAM-ICU	Confusion Assessment Method for the ICU
DTF	Diaphragmatic Thickening Fraction (the ratio of muscle thickness at expiration compared to inspiration).
FES	Functional Electrical Stimulation
FEV ₁	Forced Expiratory Volume in 1 Second
FVC	Forced Vital Capacity
Hz	Hertz – a unit of frequency required for FES
ICNARC	Intensive Care National Audit & Research Centre
ICU	Intensive Care Unit
ICU-AW	Intensive Care Unit Acquired Weakness
IL (6, 8, etc)	Interleukin
mA	Milli-amperes – a unit of current required for FES
MRC	Medical Research Council. In this context it refers to the MRC Sum Power Score, a subjective scale testing power in major muscle groups.
NMES	Neuro-muscular Electrical Stimulation

PFT	Pulmonary Function Test
SF-36	Short Form Health Survey (with 36 items)
SOFA	Sequential Organ Failure Assessment

Summary

The aim of this randomised controlled pilot trial is to assess whether a 14-day course of cycling with Functional Electrical Stimulation (FES) can prevent muscle loss and preserve muscular function in septic patients who are sedated, intubated and mechanically ventilated in the ICU, compared to a control group of patients who will receive standard daily physiotherapy.

It is known from several studies that muscle size significantly decreases early in a patient's admission to critical care with sepsis. This rapid muscle loss is likely due to a combination of disuse atrophy and systemic inflammation. Passive movement and stretching exercises performed by physiotherapists, do not appear to prevent muscle loss. Previous studies with electrical nerve stimulation have provided mixed results with respect to preserving muscle thickness. The proposed study differs from previous investigations in that we will use a cycling device which passively moves the participants legs whilst they are in bed, sedated and on a breathing machine, but with the addition of low current electrical stimulation applied on leg muscles, so that they can contract and relax in time with the movement of the cycle, thus providing "functional stimulation" rather than just electrical stimulation.

Briefly, we intend to enrol participants into the study within 48 hours of being intubated and mechanically ventilated for sepsis. Ultrasound measurements of the leg muscles, abdominal musculature and diaphragm will be performed, alongside baseline blood and urine sampling. Muscle biopsies will be taken from the leg before and after the 14-day study period. These biological samples will serve to investigate cellular mechanisms involved in muscle wasting as part of a separate translational follow-up research project.

This pilot study includes ultrasound measurements of different muscle groups and clinical outcomes in the intervention and control group and will allow us to define the most pragmatic and clinically relevant primary outcome measure for larger multi-centre trials.

Those randomised to the intervention will be in the study for 14 days. They will receive five 30 minute treatments with the device per week. Therefore in a 14 day period, they should receive 10 treatments.

Repeat ultrasound measurements will be taken on days 3, 5, 7, 10 and 14. Further blood and urine samples will be taken on days 5, 10 and 14. A second muscle biopsy will be taken on day 14.

Should patients be liberated of mechanical ventilation before day 14, they will continue in the trial, providing that they provide consent to do so.

All survivors from the trial will be invited to a follow up study 3 months from hospital discharge, where further ultrasound assessments, plus assessment of balance, strength and psychological wellbeing will be assessed.

There are two broad aims to this study. The first is to identify the clinically most useful measurement of changes in muscle size by investigating a number of muscle groups, using several indices of muscle size. This will inform us of the most suitable primary outcome for further follow-up studies.

The second aim is to assess the safety profile and feasibility of the study.

Specifically, we will determine the number of successfully completed cycling

sessions, identify logistical hurdles and patient safety risks, such as haemodynamic deterioration or accidental extubation.

Background

ICU Acquired Weakness

Intensive Care Unit Acquired Weakness (ICU-AW) is a common and potentially debilitating consequence of admission to an intensive care unit with critical illness(1). It is defined as a diffuse generalised muscle weakness without a readily identifiable cause, which occurs after developing a critical illness. Nerve conduction studies and electromyography often demonstrate both polyneuropathy and myelopathy(2). Patients with critical illness, having survived the initial acute insult, are often left with long term problems with mobility(3), which preclude independent living even five years after hospital discharge(4). These long-term sequelae represent a massive burden on both the health service and the patients' families and caregivers in the community(5).

Muscle size, expressed as either muscle thickness or muscle cross-sectional area of muscle in the limbs, can be easily tracked using bedside ultrasound methods(6). As well as size and cross-sectional area of muscles, further information, such as the arrangement of fascicles within the muscle (known as the muscle architecture)(7) and ultrasound echogenicity(8), can also be easily obtained at the bedside.

Echogenicity is helpful in determining infiltration of muscle with fat or fibrosis, which usually occurs as a result of disease or aging(9). These methods have been shown to have good reliability and accuracy in adult critically ill patients(10). Our own work has demonstrated that patients lose over 10% of their muscle thickness in the first 96 hours of mechanical ventilation, and that by 10 days, 28% of muscle thickness was lost(7). Changes to the arrangement of the fascicles within the muscle also occur, with pennation angle reducing over time, suggesting that weakness may occur

through loss of force generation as a result of smaller pennation angles. These results are in keeping with another recent study which demonstrated a 30% reduction in muscle thickness in the vastus intermedius in critically ill patients over 10 days(6) and far exceeds the previously demonstrated loss of 8% muscle thickness over five weeks bed rest in healthy volunteers(11). Ultrasound measurement of the quadriceps femoris muscle has shown a negative relationship between length of stay in critical care and muscle size during the admission(12). These structural changes are associated with loss of individual fibre area, increased loss of protein, and decreased protein synthesis, in comparison to healthy controls(13).

Physical methods of preventing and treating muscle loss

Supine cycling

Early mobilisation of patients has been shown to improve to outcomes in patients who survive critical illness(14). There are randomised control trials being carried out at present, looking at supine cycling in patients in critical care(15). Such cycling sessions can be active (entirely initiated by the patient), passive (the patient's legs are moved for them by the cycle, but no effort comes from the patient,) or assisted (where the patient makes the initial effort to move, and is then supported by the cycling device). Previously reported smaller trials have shown that the technique is safe, can be carried out in sedated patients, and can be performed safely whilst the patient is receiving intravenous infusion therapies(16). Another randomised control trial, the MoVe-ICU study, is currently in progress, examining how passive cycling in the early stages of critical illness will change the muscle morphology and ultrasound

appearance of leg muscle in these patients(17). It is, however, currently known whether early passive cycling during critical illness compared to standard treatment does improve 6-minute walk test scores in patients who survive critical illness(18).

Neuromuscular stimulation

Neuromuscular electrical stimulation (NMES) has been shown to improve muscle strength and balance in patients outside of ICU, for example stroke survivors(19). NMES used in patients after discharge from ICU resulted in no difference in muscle layer thickness, but did recover muscle strength more quickly compared to controls(20). In patients who received NMES whilst still mechanically ventilated, walking distance on discharge was greater than those receiving a sham therapy, but there was no difference in lower limb extremity muscle strength(21). The technique itself however has been shown to be safe and simple to carry out(22).

Functional Electrical Stimulation

Functional electrical stimulation (FES) also involves the use of electrically stimulating the muscles of the leg, but has been combined with a cycling device in order to carry out supine cycling exercises in patients who are still sedated. This device has been trialled in stroke patients, where it has improved muscle strength and function outcomes in a number of settings(23)(24), in comparison to passive cycling. Gait and posture have also been shown to improve after FES cycling(25).

In the critical care population, a case-control study has shown that FES with cycling improves physical function and reduces the incidence of delirium(26).

NMES and FES, whilst both using electrical current to stimulate muscle contraction, are distinct treatment modalities. NMES requires the delivery of low voltage electricity to muscles directly underneath surface electrodes which are placed on the skin(27). Studies using NMES have focussed on one muscle group per study. FES differs from NMES in that different muscle groups are stimulated in an alternating pattern. The addition of cycling allows for a muscle group to be recruited via electrical stimulation, whilst its antagonistic muscle group is relaxed. This stimulating pattern is then reversed so that the relaxed muscle is contracted, and the contracted muscle is relaxed.

There is an on-going randomised control trial, (eStimCycle – Early Rehabilitation in Critical Care, register with clinicaltrials.gov, trial identifier NCT02214823) in which patients with sepsis who are mechanically ventilated for longer than 48 hours, are being randomised to receive FES with cycling in one leg, with the other leg receiving no FES but still undergoing passive cycling, or randomised to a control group of standard physiotherapy(28). Our proposed trial will differ in the following ways:

- Both legs will be treated with FES with cycling. Comparison will be made with a separate control group receiving standard physiotherapy.
- In addition, the rectus abdominis muscle will be stimulated to assess if core muscle strength improves.
- The effects on other organ systems, including changes to the diaphragm and anterior abdominal wall muscles, will be investigated alongside changes to the lower limb. These are detailed in the methods section.

Scientific rationale

Immobility, independently of illness, leads to rapid muscle wasting. This atrophy is largely mediated through transcription of a range of “atrogenes”; including elements of the autophagic and ubiquitin proteasome systems(29). The final common path appears to be similar in disuse in volunteers and in ICU-AW patients, but in disease states additional intracellular pathways lead to atrogene transcription, resulting in a more rapid and exaggerated atrophy. Direct comparison of immobilised or bed-rested healthy individuals with ICU-AW patients are plagued with numerous confounding variables, but as a guide the former may lose 0.6% muscle size per day, whilst our data show that ICU-AW patients routinely loose about 2.5% per day(7). This figure is similar to the loss of force seen (thumb muscle) in ventilated critical care patients (30% over 14 days)(30).

In healthy individuals exercise promotes synthesis of muscle contractile proteins and supresses a range of pathways that induce atrogene transcription. In a wide range of diseases, cytokines activate the NFkB system that in turn promotes atrogenesis. The role of NFkB signalling itself is complex with both exercise and disuse reported to increase activity(31)(32)(33)(34)(35). Reactive oxygen species (ROS) are elevated in older, frail people, and this also triggers NFkB activation(36). A further pathway leading to atrogene transcription is myostatin-SMAD. This system is activated in a number of situations including ageing and critical care, but its physiological regulation is largely unknown(37). Therefore, a typical older ICU-AW patient will have a conflagration of several different intracellular pathways that lead to autophagy, mitochondrial and myofibril degradation. In this project, we will use muscle biopsies from patients undergoing FES with supine cycling, and control patients to identify the intracellular pathway changes that underlie muscle

preservation. In particular, it is important that we identify which intracellular pathway changes correlate with muscle preservation, or lack thereof, so that future intervention studies can establish whether this relationship is causal and devise novel therapeutic interventions that could be deployed independently of FES.

Cytokines have been implicated in the development of ICU-AW, and a recent study has shown that in a cohort of patients who developed ICU-AW, high levels of the interleukin (IL) -6, IL-8, IL-10 and fractalkane were found, in comparison to critically ill patients who did not develop ICU-AW(38). Raised cytokine levels may cause muscle damage through poor oxygenation of muscle(39). This may potentially be explored via a non-invasive infra-red spectroscopy technique(40).

Trial objectives and purpose

The main objective is to perform a randomised controlled pilot study to assess the effects of daily cycling with functional electrical stimulation in sedated and mechanical ventilated intensive care patients. We will measure several indices of muscle size in a number of muscles groups over a 14-day period. The second objective is to assess the feasibility of the study protocol, so that if required, study procedures can be refined for a definite randomised control trial.

Justification for a pilot study:

A wide variation in the use of electrical stimulation in the critically ill and the effects on muscle thickness has been reported in previous studies. A structured review found four studies, each assessing the effect of electrical stimulation on different muscle groups(41). Whilst two studies found that the muscle studied decreased, even with stimulation, a third study found no change to the size of the muscle, whilst a fourth demonstrated an increase in size. Furthermore, a study of critically ill patients on muscle wasting in the abdominal muscles, demonstrated that electrical stimulation slowed muscle loss in the critically ill compared to patients who did not receive it(42). The potential to increase thickness of abdominal muscle groups with neuromuscular stimulation has been demonstrated before, albeit in healthy volunteers(43). A pilot study, consisting of a number of muscle groups and measurement parameters, would allow us to refine the protocol to define the most pragmatic and clinically relevant primary outcome measure for a later definitive trial.

Research objectives:

Research objectives regarding muscle thickness and function:

1. What is the effect of a 14day course of 30 minutes cycling with functional electrical stimulation, consisting of 10 treatments over 14 days, on muscle thickness, cross-sectional area, fascicle pennation angle, fascicle length and echogenicity of the following muscles, compared to a control group receiving standard daily physiotherapy:
 - a) Vastus lateralis – all measurements
 - b) Rectus femoris, vastus medialis, vastus intermedius – cross sectional area and echogenicity
 - c) Medial head of gastrocnemius – all measurements
 - d) Rectus abdominis – Cross section area and echogenicity
 - e) Diaphragm – muscle thickness, thickening fraction and maximal excursion
2. Are such differences between treatment and control group maintained 3 months after hospital discharge?
3. Does treatment with FES and cycling affect other organ systems, as defined by requirement for renal replacement therapy, inotropic/vasopressor support, time to wean from ventilation, and incidence of delirium?
4. Do ultrasonic muscle changes correlate with biochemical alterations measured in serum and muscle tissue? The details of these changes will be described in a separate study protocol.

Research objectives to assess feasibility and patient safety:

1. How many of the 10 sessions can be successfully completed in a 14-day period? Successful completion will comprise at least 20 min of cycling activity.
2. What are the reasons for missed or incomplete treatment sessions?
3. What is the incidence of adverse effect such as haemodynamic changes (blood pressure or heart rate) of more than 20% baseline, central or arterial line dislodgement, accidental extubation or falls?

Study design

We intend to perform a randomised control pilot trial with two arms: 1. Patients receiving standard physiotherapy will serve as a control group, 2. Patients undergoing FES plus supine cycling 5 times per week. In a number of muscle groups, muscle thickness will be measured with ultrasound to determine the clinically most useful index to describe changes in muscle size during treatment with 10 sessions of FES with cycling over a 14-day period.

Subject selection

Patients will be recruited in the Intensive Care Unit of the Royal Liverpool University Hospital. All patients will be over 18, and have a critical illness that requires mechanical ventilation with an initial period of sedation. This study will focus on patients with a definite or suspected case of sepsis from any source.

Sepsis has been recently redefined as: “*Life threatening organ-dysfunction caused by dysregulated host response to infection*” whilst septic shock has become a subset

of sepsis, defined as: “*circulatory and cellular/metabolic dysfunction associated with a higher risk of mortality*”(44).

For the purposes of this study, a patient will be regarded as septic if they have evidence of infection-related organ failure (e.g. sepsis-associated coagulopathy, altered mental state, cardiovascular dysfunction, acute kidney injury, and altered liver function) and require invasive mechanical ventilation with either definite or suspected evidence of infection. This is to allow prompt treatment with FES rather than waiting for a positive microbiological result to be obtained.

Within the definition of sepsis “from any source” a list of following is illustrative but not exhaustive:

- Urogenital sepsis (including urosepsis, pyelonephritis, endometritis and chorioamnionitis)
- Pneumonia (including community acquired, hospital acquired, and aspiration pneumonia. Ventilator associated pneumonia would be excluded.)
- Neurological infections such as encephalitis and meningitis.
- Cellulitis, osteomyelitis and infections of soft tissue NOT affecting the lower limb.
- Surgical infections, including post-operative laparotomy with evidence of peritoneal soiling, and evidence of infection prior to the operation, in patients who require 2 or more organ system support after the operation.
- Intra-abdominal sepsis, including biliary sepsis, hepatitis, and acute pancreatitis. In the case of acute pancreatitis, evidence of infection is required to fulfil the criteria. Acute pancreatitis with sterile tissue/fluid samples would not be suitable.

Exclusion criteria include:

- Patients under 18
- Patients who decline consent
- Pregnancy
- Neuromuscular disease
- Rhabdomyolysis
- Lower limb trauma
- Patients unlikely to survive to 96 hours post admission
- Consent unobtainable within 48 hours of admission
- Morbid obesity (BMI>40).
- Presence of a pacemaker or Implantable Cardiac Defibrillator (ICD).
- Unlikely to be mechanically ventilated for more than 48 hours.

Subject recruitment

Patients will be mechanically ventilated and sedated and therefore unable to consent to the trial. The patient's next of kin will be approached to act as a consultee, to provide consent for the patient to take part in the study. If the patient regains capacity to consent for themselves, they will be informed about their participation in the study and retrospective consent will be sought.

The process for taking consent from the next of kin is as follows:

Patients will be screened every morning by the research nursing team to identify potential patients who meet the inclusion criteria. Eligible patients will be discussed with the medical team that day to assess if the medical condition or comorbidities

preclude recruitment into the trial (for example, the medical team have decided to withdraw care).

The next of kin will be approached by a member of the research team. The aims of the study and a discussion of what would happen to their relative during the trial will take place, and an information leaflet will be given to the next of kin. At this point, there is no obligation to sign a consultee declaration form. The next of kin will be given time to think about whether they want their relative to take part, and they can discuss the study with other members of the family.

Once the next of kin has decided to provide consent, they will sign a consultee declaration form, acting as a consultee on behalf of their relative. Should they later decide to withdraw this declaration, the patient will be withdrawn from the study, and all samples and data will be disposed of.

Once the patient regains capacity, they will be approached by the research team, explaining the involvement in the study so far, the aims of the study, and what will happen if they agree to continue in the study. They will again be given time to think about whether they want to continue in the study, and will be given an information leaflet to read. There is no time limit on this, and once ready, the patient will have to provide retrospective consent by signing the appropriate retrospective consent form. Should they decide they do not want to take part in the study, all further cycling sessions would be cancelled. An added option has been incorporated into this form, in the event that the patient decides they do not want any more cycling sessions, but that we can continue to store and use blood, urine and muscle biopsy samples, and that no further samples will be taken. In the event that they do not consent to us using pre-existing samples, and they do not want any further cycling sessions, then

all samples will be destroyed. This includes the deletion of ultrasound images from any storage medium.

Where a next of kin cannot be contacted within 36 hours of admission, a professional consultee declaration by the patient's clinical team will be considered to allow recruitment into the study within the 48h time frame. The senior clinician in the medical team will be provided with an information leaflet, and when ready, will be given a Nominated Professional Consultee Declaration form to sign.

Patients for whom a consultee declaration form cannot be signed within 48h after intubation, will need to be excluded from the trial.

Trial interventions and investigations

The day on which a valid consultee declaration is obtained, will be classified as Day 0. Within 24 hours, patients will receive an ultrasound of the vastus lateralis to assess muscle thickness and fascicle pennation angle, and ultrasounds of the four heads of the quadriceps (rectus femoris, vastus lateralis, medialis and intermedius), medial head of gastrocnemius, rectus abdominis and diaphragm. Blood and urine samples will be taken from pre-existing arterial (or central venous) lines and urinary catheters. A percutaneous muscle biopsy will also be taken under sedation and local anaesthesia from the vastus lateralis muscle. All samples will be obtained on study day 1 before the intervention is started.

Ultrasound scans will be repeated on days 3, 5, 7, 10 and 14. Our work and previous publications suggest that significant losses in muscle thickness can be detected by

ultrasound as early as day 5, and are more pronounced by day 10. Further blood and urine samples will be taken on days 5, 10 and 14.

Ultrasound scanning of the following muscles will take place on day 1:

- Vastus lateralis
- Rectus femoris Vastus lateralis, intermedius and medialis (cross section area).
- Medial head of gastrocnemius
- Diaphragm
- Rectus abdominus

All ultrasound scans will be taken with the patient lying supine. Vastus lateralis and rectus femoris imaging will be performed with the limbs straight and in contact with the bed. Gastrocnemius scans will be taken with hip and knee flexed so that the foot is in contact flat with the bed. These methods have been used in our previous study(7) and have been cited in other work(45). Diaphragmatic ultrasound will include diaphragmatic thickness, Diaphragmatic Thickening Fraction (DTF) and diaphragmatic excursion on both sides according to standard procedures.

Muscle biopsies will be taken percutaneously under local anaesthetic, on days 1 and 14 only.

After the initial samples have been taken, the patient will be randomised using a computer-generated randomisation system, to receive either FES plus cycling or standard physiotherapy. A computer-based block randomization system will be generated using R (randomizeR: An R Package for the Assessment and Implementation of Randomization in Clinical Trials). Allocation concealment will be

guaranteed by management of the randomization table by an external company not involved in the conduct of the study (MoRe Data GmbH, Giessen, Germany).

Patients receiving FES plus cycling will receive 30 minutes of FES cycling on 5 days per week. Other studies have applied between 20 -50 minutes of this exercise. The device to be used is the Restorative Therapies (RT) 300 Supine with FES system, plus a 12 channel SAGE stimulator (Restorative Therapies Ltd, Baltimore, Maryland, USA). Surface electrodes will be placed on the quadriceps, hamstrings and calf muscles of both legs, with an additional electrode placed across the rectus abdominis muscle. The aim of the electrical stimulation is to produce visible contractions in each muscle group, without causing pain. If a contraction cannot be seen, treatment will continue, but with regular palpation of the muscle groups to feel for contraction. This will be done every 5 minutes throughout the intervention period. In line with eStimCycle study, a maximum current of 140mA, pulse duration of 250-500 microseconds, frequency of 40 Hz and pedal cadence of 30-45 revolutions per minute will be applied. The intervention will be performed within 24h after consent has been obtained and will be classified as study day 1. Study day 1 has to occur within 72 hours of intubation. Patients will receive the intervention on 5 days per week. There will be one cycling device on the Intensive care Unit, which can be used for 6-8 cycling sessions per day. Hence a total of up to 16 patients can be included at any given time point (6-8 patients in the interventional arm, 6-8 patients in the control group).

Patients will be fully monitored during the study, with standard ICU equipment, including electrocardiogram, heart rate, arterial blood pressure, end-tidal carbon dioxide tension and haemoglobin oxygen saturation. Heart rate variability will also

be measured using a skin sensor device (Isansys Lifecare Ltd, Abingdon, Oxfordshire, UK).

All patients randomised to the intervention arm will have cycling sessions with FES for 14 days. They will receive 5 sessions per week, and so should receive 10 sessions over a two week period. Should the patient be liberated from mechanical ventilation, they will carry on receiving cycling with FES until day 14. In the unlikely event that they cannot tolerate FES once extubated, the intervention will be stopped. Based on discussions with the manufacturer, we expect that less than 10% of patients would be unable to tolerate FES whilst extubated, however, we wish to confirm this statement as part of this pilot trial to inform future studies..

All patients, regardless of intubation status, will have no further sessions of FES after day 14. No further ultrasound scans, blood or urine tests will be conducted after day 14.

Ultrasound scans performed on day 14 will serve as the main comparator to baseline investigation.. In patients who failed to tolerate the intervention, ultrasound measurements will give an opportunity to assess if muscle is lost whilst not receiving FES, but still critically ill.

Patients with a tracheostomy allocated to intervention will also receive FES with cycling until day 14.

On day 14, in patients that are awake and suitably co-operative, assessment of strength using both hand grip dynamometry and the MRC Sum power score will be conducted by blinded physiotherapists.

Follow up testing will occur at 3 months post hospital discharge. This will include:

- Ultrasound of the 4 heads of the quadriceps and gastrocnemius muscles.
- Ultrasound of the diaphragm muscles.
- Performance of balance and gait testing using an electronic pressure detecting plate.
- Performance of a 6-minute walk test.
- MRC sum power test
- Handgrip strength using portable dynamometry
- Assessment of quality of life, using the SF-36 scale - The SF-36 questionnaire is commonly used for follow-up in research studies involving critically ill patients and is routinely used in our follow-up clinics, to which patients are invited to attend 3 months after ITU discharge. If patients are not available to attend the follow-up clinics, an attempt will be made to obtain the SF-36 information via telephone interview. Patients will have the opportunity to decline participation.

There will be no procedure for breaking the codes for randomisation, as the study team and the patient cannot be blinded to the treatment allocation.

Patient end-points

A patient will be removed from the study if:

- A relative withdraws consent on behalf of the participant.
- The patient, after regaining capacity, withdraws consent.

- There is difficulty in complying with treatment with more than two treatment sessions per week being missed (e.g. due to haemodynamic instability).
Criteria for a patient to not receive a cycling session are in line with the CYCLE trial¹⁵.

Data to be collected

- Muscle cross sectional area, muscle thickness, muscle fascicle length, pennation angle and echogenicity in the vastus lateralis and medial head of gastrocnemius, change in muscle thickness and echogenicity of the rectus abdominus, diaphragm and change in cross sectional area of the rectus femoris and the three vastus muscles. These will be measured on days, 1, 3, 5, 7, 10, and 14.
- Relationship between changes in muscle thickness and expression of blood and muscle inflammatory markers.
- Measures of balance and posture using a specialised balance board in patients who survive to discharge from ICU. This will be tested 3 months after hospital discharge.
- Long term outcome measures, including ultrasound measurement of ALL muscle groups, and tests of balances and gait, re-testing of the MRC Sum Power Score, and 6-minute walk test. Quality of life assessment will be conducted using the SF36 questionnaire.
- Ultrasonographic changes in diaphragmatic thickness, DFT and diaphragmatic excursion
- Incidence of delirium, as assessed by the CAM-ICU method.

- Data collected from routine monitoring, such as heart rate, heart rate variability, haemoglobin oxygen saturation and end tidal carbon dioxide tension.

Other data collected

To answer the secondary research questions, the following data will also be collected:

- Age, sex, weight, height, and Body Mass Index (BMI)
- Admission APACHE II, ICNARC and SOFA scores
- Routine blood results, including full blood count, urea and electrolytes, liver function tests, clotting screen and arterial blood gases.
- A daily diary of standard physiotherapy received, time taken, and treatment received.
- A daily diary of FES and cycling, including time spent cycling.
- Blood glucose measurements.
- Total infusion volumes, including inotropes/vasopressors, insulin, sedative agents, intravenous fluids, nasogastric feed and total parenteral nutrition.
- Documented evidence of delirium, as assessed by the CAM-ICU method.
- Ventilator settings, including inspired oxygen concentration, and time to wean off mechanical ventilation.
- Use of renal replacement therapy, mode of replacement and indication for replacement.
- Microbiological samples for culture.

- A “safety log” of incidents and injuries sustained in the course of using FES with cycling.
 - Dislodgement of arterial lines, central lines, urinary catheter and endotracheal tubes.
 - Haemodynamic changes including changes in blood pressure or heart rate of more than 20% of baseline.
 - Falls
 - Other injury to staff or patients
 - Death
- Dose of cardiovascularly active drugs (inotropes, anti-arrhythmics, vasopressors)

Further laboratory studies

As well as expression of cytokines and histones in both blood and muscle samples, muscle biopsies will be used to conduct single fibre contractility studies. Using the blood and muscle cytokine and histone levels, we can assess if contractility in these fibres is reduced in patients who have greater levels of inflammatory markers.

Haematoxylin and eosin (H&E) staining will also be performed, to assess nuclei count, extra-cellular matrix production and muscle area.

Blood samples will be analysed by ELISA for a panel of cytokines (including muscle specific cytokines, ‘myokines’) and biopsies will be subject to RNA-sequencing to allow analysis of genome differentially transcribed genes independently of bias. Key members of differentially expressed gene pathways will be verified by qPCR and Western blots. To investigate whether differentially expressed genes result from

change in cytokine abundance, we will simulate this system *in vitro*. Cultured human muscle cells will be exposed to the observed differentially expressed cytokines and RNA-sequenced.

All laboratory investigations on samples obtained in this trial are part of a different funding and research application.

Statistical analysis

In general, sample size calculations are not required for pilot studies. We chose to continue recruitment until 18 patients in each group have reached day 14 of the study. This design will allow us to calculate a realistic drop-out rate for future trials and thus assess feasibility. We will use the inclusion/exclusion criteria we anticipate using in follow-up trials. With 18 patients in each arm completing the study until day 14, we anticipate that useful information about the feasibility, the safety and the changes in muscle thickness can be obtained.

Although not the primary aim of this project, the results from this pilot study may be used to generate data for sample size calculations for larger follow-up trials.

Our previous non-interventional study showed a 50% drop out between days 1 and 10. Therefore, we anticipate recruitment of 36 patients per arm to achieve completion of the trial until day 14 in 18 patients.

The following estimates were considered for drop-out: Loss of 14-day follow-up due to death (10%), discharge from Intensive Care or High-Dependency Units before day 10 (10%), inability to tolerate the intervention (20%), decline to continue in the trial after regaining capacity (10%).

A detailed breakdown of all measurements performed at different time points and the proposed statistical approach is listed in Appendix C.

In all statistical analyses, a p-value of less than 0.05 will be considered statistically significant. For multiple testing the Bonferroni-Holm correction will be applied.

Blinding

Due to the nature of the study, it is not possible to blind the participants or the investigators. However, ultrasound images will be saved as numbered images, with no detail as to which group the images come from. Measurement of muscle thickness will be measured on a computer rather than at the bedside with ultrasound machine, so that the measurements are performed blinded.

2.2.9 Ethical considerations

Considerations related to subject selection and recruitment have been described already.

Blood samples and urine samples shall be taken from the patients indwelling arterial/central lines and urinary catheters. Surplus blood samples from blood taken for routine clinical purposes will be collected daily from the Clinical Chemistry Department. Should an arterial line or central line be unavailable, blood will be obtained from venepuncture, if possible as part of a daily routine venepuncture to minimise pain and infection risk. Urine sampling will be performed via an indwelling urinary catheter or via collection a urine sample at the bedside.

Muscle biopsies taken percutaneously may cause pain, skin infection, muscle and fat infection and bleeding. The risk of skin infection in one trial is 1 in 800(46), and serious bleeding reported in 1 in 5000 cases(47).

End of study, dissemination and archiving

The study is expected to start on August 1st 2018. The study is expected to run for 2.5 years, ending on the 1st February 2021. The recruitment period is expected to last 12-18 months with expected recruitment of 2 patients/week. Data analysis and final report will take 6 months. The suggested study period will allow a 3 month follow up appointment, within the 2.5 years. An interim analysis will take place after 50 patients will have been recruited.

The study will be ended early in the event that:

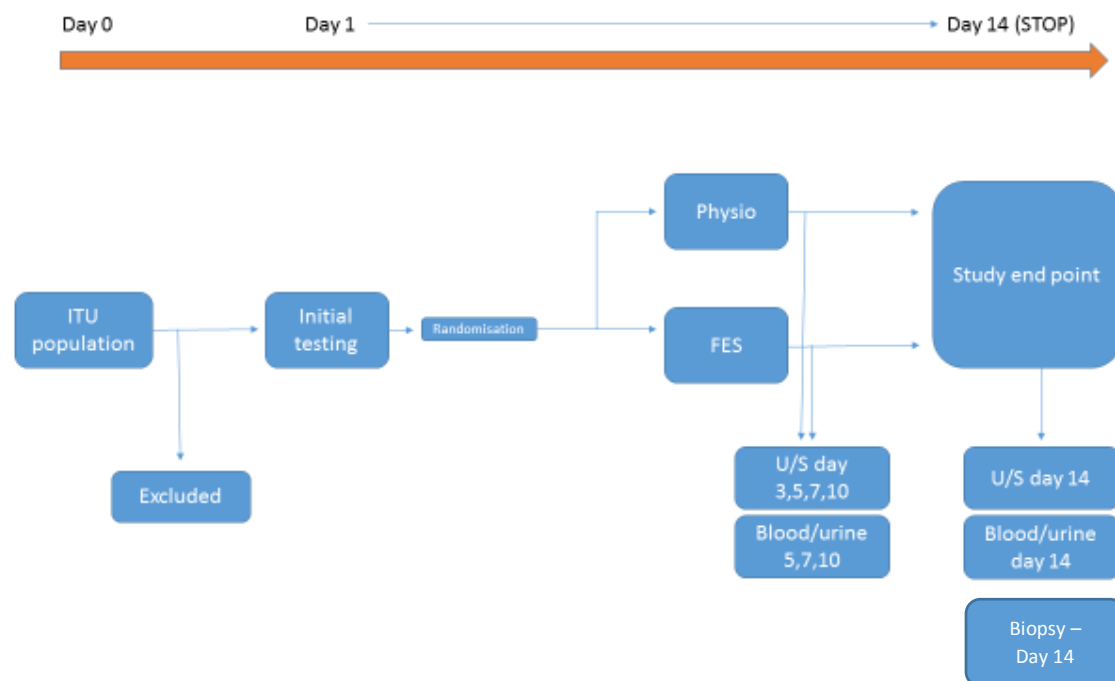
- Inability to recruit patients within 3 months after opening the trial.
- Serious adverse effects associated with the trial device
- Decision of the Data management committee after interim analysis
- The study sample is completed

Data will be presented at local, national and international conferences, as well as for publication in journals. Patients who took part in the study can have their results discussed at the 3 months follow up appointment, and will also be sent copies of any publication on request.

After data analysis is completed, all study material will be archived using an approved archiving company, in this instance Manchester Retrievals.

Appendix A – Study Flow Diagram

Recruitment, randomisation and groups



Notes:

Patients may be extubated between day 1 and 14. If they are, they will continue to receive FES with cycling whilst awake. In the unlikely event that they cannot tolerate this, they will receive passive cycling without electrical stimulation until day 14.

Even if a patient is still intubated by day 14, the intervention will stop at day 14.

Follow up testing will occur at 3 months post hospital discharge.

Time scale of investigations and interventions

Procedure/Test	Day 0	Day 1	Day 3	Day 5.	Day 7	Day 10.	Day 14	F/U 3/12
Consent	x							
Randomisation		x						
Start Intervention		x						
Urine		x		x		x	x	
Muscle biopsy		x					x	
Blood		x		x		x	x	
U/S		x	x	x	x	x	x	x
Functional testing								x
Balance Board								x
Psychological								x

Heart monitoring and haemoglobin oxygen saturations will take place during cycling sessions.

Cycling will be daily, for 5 times per week.

Appendix B – The CYCLE Trial Exclusion Criteria

A. Daily exemption criteria for in-bed cycling

Cycling or physiotherapy (PT) sessions will not occur if any of the following conditions are present:

1. An increase in vasopressor/inotrope of more than 25% within past 2 h
2. Active myocardial ischaemia, or unstable/uncontrolled arrhythmia after discussion with the intensive care unit (ICU) team.
3. Mean arterial pressure (MAP) <60 or >110 mm Hg or out of range for this patient within the past 2 hours.
4. Heart rate <40 or >140 bpm within the past 2 hours.
5. Persistent SpO₂ <88% or out of range for this patient within the past 2 hours.
6. Neuromuscular blocker within past 4 hours.
7. Severe agitation (Richmond Agitation and Sedation Scale >2 (or equivalent)) within past 2 hours.
8. Uncontrolled pain.
9. Change in goals to palliative care.
10. Team perception that in-bed cycling or therapy is not appropriate despite absence of above criteria (e.g., active major haemorrhage from any site, acute peritonitis, new incision or wound precluding cycling, new known/suspected muscle inflammation (e.g., rhabdomyolysis)).
11. Patient or proxy refusal.

B. Criteria to terminate in-bed cycling or routine PT

Cycling or routine PT will stop if the following occurs:

1. Concern for myocardial ischaemia or suspected new unstable/uncontrolled arrhythmia.
2. Unplanned extubation
3. Physiotherapist perceives continuing cycling or routine PT is not appropriate, for example,
4. Respiratory—sustained O₂ desaturation <88%; marked ventilator dysynchrony.

5. Cardiovascular—sustained symptomatic bradycardia (<40 bpm), tachycardia (>140 bpm), hypotension (MAP <60 mm Hg) or hypertension (MAP >120 mm Hg).
6. Catheter or tube dislodgement or severe patient agitation.
7. ICU physician, patient or proxy requests termination of session.

Appendix C – Proposed measurements and statistical test

An overview of all measurements and planned statistical analysis is presented in the table below. A p-value of less than 0.05 will be considered statistically significant.

Appendix C – Proposed measurements and statistical test

An overview of all measurements and planned statistical analysis is presented in the table below. A p-value of less than 0.05 will be considered statistically significant.

Due to multiple comparisons, the Holm–Bonferroni method is used to control the group-wise error rate.

In all cases, a p-value of <0.05 after Holm–Bonferroni correction will be considered statistically significant.

Category	Measurement	Units	Time of Measurement	Summary Measure	Hypothesis to be tested (H0-hypothesis)	Statistical Test	NOTES
Patient Demographics	Age	Years	On admission/consenting	Mean and SD	There is no difference between intervention and control group	Unpaired t-test	
	Height	Metres	On admission/consenting	Mean and SD	There is no difference between intervention and control group	Unpaired t-test	
	Weight	Kilograms	On admission/consenting	Mean and SD	There is no difference between intervention and control group	Unpaired t-test	
	BMI	kg/m ²	On admission/consenting	Mean and SD	There is no difference between intervention and control group	Unpaired t-test	
	Sex	Count	On admission/consenting	Count and percentage of group	There is no difference between intervention and control group	Chi-square test	
	APACHE II score	Arbitrary Points	On admission/consenting	Mean and SD	There is no difference between intervention and control group	Unpaired t-test	
(Vastus lateralis)	Muscle thickness	Centimetres	Day 1, 10	Mean change in thickness (cm), SD	There is no difference between intervention and control group	Unpaired t-test	
				Mean % change in thickness, SD	There is no difference between intervention and control group	Unpaired t-test	
				Skew, Kurtosis	The data are normally distributed	Shapiro-Wilk	
Ultrasound	Fascicle pennation angle	Degrees	Day 1, 3, 5, 7, 10, 14	Mean change in angle (degrees), SD	There is no difference between intervention and control group	Repeated measure analysis	
				Mean % change in angle, SD	There is no difference between intervention and control group	Repeated measure analysis	
(Vastus lateralis, medial head of gastrocnemius)	Fascicle length	Centimetres	Day 1, 3, 5, 7, 10, 14	Mean change in length (cm), SD	There is no difference between intervention and control group	Repeated measure analysis	
		(Derived by trigonometry)		Mean % change in length, SD	There is no difference between intervention and control group	Repeated measure analysis	FL = thickness / sin (Pennation angle)
Medial head of gastrocnemius	Muscle thickness	Centimetres	Day 1, 3, 5, 7, 10, 14	Mean change in thickness (cm), SD	There is no difference between intervention and control group	Repeated measure analysis	
				Mean % change in thickness, SD	There is no difference between intervention and control group	Repeated measure analysis	
Rectus femoris, Vastus lateralis Intermedius and Medialis	Cross sectional area	Square Centimetres	Day 1, 3, 5, 7, 10, 14	Mean change in area (cm ²), SD	There is no difference between intervention and control group	Repeated measure analysis	
				Mean % change in area, SD	There is no difference between intervention and control group	Repeated measure analysis	
Rectus Abdominis	Cross sectional area	Square Centimetres	Day 1, 3, 5, 7, 10, 14	Mean change in area (cm ²), SD	There is no difference between intervention and control group	Repeated measure analysis	
				Mean % change in area, SD	There is no difference between intervention and control group	Repeated measure analysis	
Diaphragm	Thickness	Centimetres	Day 1, 3, 5, 7, 10, 14	Mean change in thickness (cm), SD	There is no difference between intervention and control group	Repeated measure analysis	
				Mean % change in thickness, SD	There is no difference between intervention and control group	Repeated measure analysis	
All muscles	Echogenicity	Arbitrary, scale from 0-1	Day 1, 10	Change in echogenicity, SD	There is no difference between intervention and control group	Unpaired t-test	
Diaphragm	Thickening Fraction	Percentage	Day 1, 3, 5, 7, 10, 14	Mean change in Fraction (%), SD	There is no difference between intervention and control group	Log Rank test	
Diaphragm	Maximal Excursion	Centimetres	Day 1, 3, 5, 7, 10, 14	Mean change in excursion (cm), SD	There is no difference between intervention and control group	Log Rank test	
Measures of correlation	Change in muscle thickness on Day 10 related to age/weight/baseline muscle thickness	Centimetres/s/s/yr/kg/cm	Losses by Day 10	Correlation coefficient, R	There is no correlation between muscle thickness on Day 10 and age/weight/baseline muscle thickness between intervention and control group	Pearson's test	
Biochemical testing	Muscle Biopsy	TBC	Day 1, Day 10	TBC	TBC	TBC	
	Blood/Urine	TBC	Day 1, 5, 7, 10	TBC	TBC	TBC	
Follow up measures	Hand grip	Newtons	At 3 months / U appointment	Mean difference in force (N), SD		unpaired t-test	
	Dynamometry Force, flexion at the knee	Newtons	At 3 months / U appointment	Mean difference in force (N), SD		unpaired t-test	
	6 minute walk test	Metres	At 3 months / U appointment	Mean difference in distance (metres), SD		unpaired t-test	
	Ultrasound Vastus lateralis	Centremeters	At 3 months / U appointment	Mean difference in thickness (cm), SD		unpaired t-test	
	SF-36 score	Arbitrary points	At 3 months / U appointment	Mean difference in points, SD		unpaired t-test	
	Balance	Average sway	At 3 months / U appointment	Mean difference in sway, SD		unpaired t-test	
MRC Sum of Power score	Total score	Arbitrary from 0-36 points	At 3 months / U appointment	Mean difference in score, SD		unpaired t-test	
Other	Incidence of delirium	Count and %	From days 1-14	Percentage difference		Chi-square test / Fisher's exact test	
	Incidence of feeding RRT	Count and %	From days 1-14	Percentage difference		Chi-square test / Fisher's exact test	
	Safety of tubes/lines pulled	Count and %	From days 1-14	Percentage difference		Chi-square test / Fisher's exact test	

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